Supplementary Information

Phage Anti-Pycsar Proteins Efficiently Degrade β-Lactam Antibiotics

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**Figure S1.** Aligned metal-binding residues of Apyc orthologs and representatives of the MBL-fold superfamily. Amino acid sequences from various functional subgroups of the MBL-fold superfamily were aligned using structure-guided sequence alignment with MAFFT-DASH (L-INS-i) [55,56]. Columns corresponding to residues comprising the α (H64, H66, H154) and β (D68, H69, H233) metal-binding sites, and the metal-bridging position (178) were extracted for representative sequences. ApycGoe3 and ApycGrass display the same metal binding motifs as Apyc1 and much of the broader superfamily, and possess the bridging aspartate residue absent in true metallo-β-lactamases (*i.e.*, the B1, B2, and B3 MBLs).

**Table S1**: Catalytic parameters of representative members of each Ambler class of β-lactamases. Classes A, C, and D are SBLs, while Class B (inclusive of the B1, B2, and B3 subgroups) are MBLs. Units of kcat, KM, and kcat/KM are s-1, μM, and s-1 mM-1. aYong *et al.* [37], bBottoni *et al.* [57], cHorsfall *et al.* [58], dSegatore *et al.* [59], e Bebrone *et al.* [60], f Venkatachalam *et al.* [61], gDe Wals *et al*. [62], hPoirel *et al.* [63], iRobin *et al.* [64], jMarcoccia *et al.* [65], h Chiou *et al*. [66], i Mammeri *et al.* [67], j Mazzariol *et al.* [68], k Lenfant *et al.* [69]. N.H. - no hydrolytic activity detected. | N.D. – not determined.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **TEM-1** **(Class A)** | **NDM-1a** **(Class B1)** | **CphA** **(Class B2)** | **AIM-1c** **(Class B3)** | **AmpC (*E. coli K12*)** **(Class C)** | **OXA-48** **(Class D)** |
| **Substrate** | **kcat** | **KM** | **kcat/KM** | **kcat** | **KM** | **kcat/KM** | **kcat** | **KM** | **kcat/KM** | **kcat** | **KM** | **kcat/KM** | **kcat** | **KM** | **kcat/KM** | **kcat** | **KM** | **kcat/KM** |
| **Penicillins** |
| Penicillin G | 1660g | 62g | 26,774g | 11 | 16 | 680 | 3d/0.03e | 630d/870e | 4.8d/0.034e | 778 | 31 | 25,000 | 45 | 4.4 | 10,227 | 245h | 40h | 6’100h |
| Ampicillin | 1450f | 71f | 20,422f | 15 | 22 | 660 | <0.01e | 2’500e | <0.004 | 594 | 41 | 14,000 | 4.2 | 3.5 | 1,200 | 340h | 5’200h | 65h |
| Carbenicillin | 120k | 14k | 8,571k | 108j | 285j | 379j | 10d | 500d | 20d | - | - | - | 0.002j | 0.1j | 20j | 311k | 57k | 5’456k |
| **Carbapenems** |
| Meropenem | N.H. | N.H. | N.H. | 12 | 49 | 250 | 3,100b/53d | 1,600b/250d | 1,940b/212d | 1’000 | 163 | 6,100 | - | - | - | 0.1h | 200h | 0.5h |
| Imipenem | N.H. | N.H. | N.H. | 20 | 95 | 210 | 460b/140d/1,200e | 110b/86d/340e | 4,180b/1,627d/3,529e | 1’700 | 97 | 18,000 | 0.09i | 32’000i | 0.0028i | 2h | 14h | 145h |
| **Cephalosporins** |
| Cephalothin | 77g | 180g | 427g | 4 | 10 | 400 | - | - | - | 529 | 38 | 14,000 | 300 | 42 | 7,142 | 3h | 120h | 150h |
| Cefuroxime | <0.1i | N.Di | N.Di | 5 | 8 | 610 | - | - | - | 292 | 29 | 10,000 | 0.15j | 0.15j | 1,000j | - | - | - |